

WHAT IS CLAIMED IS:

1. A recombinant RNA molecule comprising a binding site specific for an RNA-directed RNA polymerase of a negative strand RNA virus, operatively linked to a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation.

2. The recombinant RNA molecule of Claim 1 in which the polymerase binding site comprises the polymerase binding site contained in the 3'-noncoding flanking sequence of an influenza genome vRNA segment.

3. The recombinant RNA molecule of Claim 1 in which the polymerase binding site comprises the terminal 12 nucleotides of the 3'-terminus of an influenza genomic segment.

4. The recombinant RNA molecule of Claim 1 in which the 3'-noncoding viral sense flanking sequence of influenza comprises the following sequence:

5'-CCUGCUUU<sup>C</sup><sub>U</sub>GCU-3'

5. A recombinant RNA molecule comprising a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to a 3'-noncoding flanking sequence of an influenza vRNA containing the viral polymerase binding site, and to a 5'-noncoding flanking sequence of an influenza vRNA.

6. The recombinant RNA molecule of Claim 5 in which the 5'-noncoding flanking sequence of an

influenza vRNA comprises the first 22 nucleotides of the 5'-terminus of an influenza genomic segment.

7. The recombinant RNA molecule of Claim 5 in which the 5'-noncoding flanking sequence of an influenza vRNA comprises the following sequence:  
5'-AGUAGAAACAAGGGUGUUUUUU-3'.

8. A recombinant RNP comprising the recombinant RNA molecule of Claim 1 complexed with the purified RNA-directed RNA polymerase.

9. A recombinant RNP comprising the recombinant RNA molecule of Claim 2 complexed with a purified influenza viral polymerase.

10. The recombinant RNP of Claim 9 in which the influenza viral polymerase is obtained from RNPs fractionated by centrifugation on a CsCl gradient, in which the purified influenza viral polymerase is isolated from the region of the gradient correlating to 1.5 to 2.0 M CsCl.

11. A recombinant RNP comprising the recombinant RNA molecule of Claim 5 complexed with a purified influenza viral polymerase.

12. The recombinant RNP of Claim 11 in which the influenza viral polymerase is obtained from RNPs fractionated by centrifugation on a CsCl gradient, in which the purified influenza viral polymerase is isolated from the region of the gradient correlating to 1.5 to 2.0 M CsCl.

13. A chimeric virus comprising influenza virus containing a heterologous RNA sequence comprising the

reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to an influenza viral polymerase binding site.

5           14.    The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 1 of influenza virus.

10           15.    The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 2 of influenza virus.

15           16.    The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 3 of influenza virus.

20           17.    The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 4 of influenza virus.

20           18.    The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 5 of influenza virus.

25           19.    The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 6 of influenza virus.

30           20.    The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 7 of influenza virus.

35           21.    The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 8 of influenza virus.

22. A chimeric virus comprising influenza virus containing in addition to its eight genomic segments an additional RNA segment containing a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal  
5 initiation of translation, operatively linked to an influenza viral polymerase binding site.

23. The chimeric virus of Claim 22 further  
10 containing a selectable coding sequence such that the bicistronic mRNA coding sequence is stably expressed.

24. A chimeric virus comprising a negative strand RNA virus containing a heterologous RNA  
15 sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to a polymerase binding site of the negative-strand RNA virus.

20 25. The chimeric virus of Claim 24 further containing a selectable coding sequence such that the bicistronic mRNA coding sequence is stably expressed.

25 26. A recombinant DNA molecule encoding the recombinant RNA molecule of Claim 1 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

30 27. A recombinant DNA molecule encoding the recombinant RNA molecule of Claim 2 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

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28. A recombinant DNA molecule encoding the recombinant RNA molecule of Claim 5 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

5 29. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of Claim 8 so that the heterologous gene is expressed in the culture.

10 30. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of Claim 9 so that the heterologous gene is expressed in the culture.

15 31. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of Claim 11 so that the heterologous gene is expressed in the culture.

20 32. A method for producing a chimeric negative-strand RNA virus, comprising culturing a host cell transfected with the recombinant RNP of Claim 8 and infected with a parental strain of the negative strand RNA virus, and recovering the chimeric virus from the culture.  
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30 33. A method for producing a chimeric influenza virus, comprising culturing a host cell transfected with the recombinant RNP of Claim 9 and infected with a parental strain of influenza, and recovering the chimeric influenza virus from the culture.

35 34. A method for producing a chimeric influenza virus, comprising culturing a host cell

transfected with the recombinant RNP of Claim 11 and infected with a parental strain of influenza, and recovering the chimeric influenza from the culture.

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